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Authors

Palmer, J.
Moses, V.

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University of California

Ernest O. Lawrence Radiation Laboratory

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INVOLVEMENT OF THE LAC REGULATORY GENES IN CATABOLITE REPRESSION IN ESCHERICHIA COLI

J. Palmer and V. Moses

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Involvement of the Lac Regulatory Genes in Catabolite Repression in Escherichia coli

By J. Palmer and V. Moses

Lawrence Radiation Laboratory
University of California
Berkeley, California
U.S.A.

1. Acute transient catabolite repression of β-galactosidase synthesis, observed when glucose is added to glycerol-grown cells of Escherichia coli (Moses & Prevost, 1966), requires the presence of a functional operator gene (o) in the lactose operon. Total deletion of the operator gene abolished acute transient repression, even in the presence of a functional regulator gene (i). 2. Regulator constitutives (i^-) also show transient repression provided that the operator gene is functional. Regulator deletion mutants (i^{del}), with which to test specifically the role of the i gene, have not so far been available. 3. The above mutants, showing various changes in the lactose operon, show no alteration in the effect of glucose on induced tryptophanase synthesis. Glucose metabolism, as measured in terms of the release of $^{14}CO_2$ from [$^{14}C_1$]glucose and [$^{14}C_6$]glucose, also showed no differences between strains exhibiting or not exhibiting transient repression. This suggests no change in the operation of the pentose phosphate cycle, a metabolic activity known to be of paramount importance for glucose repression of β-galactosidase synthesis (Prevost & Moses, 196). 4. Chronic, permanent repression by glucose of β-galactosidase synthesis (less

severe in degree than acute transient repression) persists in strains in which transient repression has been genetically abolished. Constitutive alkaline phosphatase synthesis, which shows no transient repression, also demonstrates chronic permanent repression by glucose.

5. Chloramphenicol repression also persists in mutants with no transient repression, and also affects alkaline phosphatase. It is suggested that chronic, permanent repression and chloramphenicol repression are non-specific, and that they do not influence β-galactosidase synthesis via the regulatory system of the lactose operon.

The rate of synthesis of β-galactosidase in Escherichia coli is subject to at least two physiological control systems: induction-repression, as described by Jacob & Monod (1961), and catabolite repression (Magasanik, 1961). Catabolite repression, in general, affects only those inducible enzymes which are responsible for providing the cell with carbon skeletons and energy. The synthesis of such enzymes is repressed whenever the total rate of consumption of carbon sources exceeds the rate required for biosynthesis (Neidhardt, 1960). Thus, restriction of the rate of catabolism (e.g. by the rate-limiting addition to cells of the carbon source) tends to derepress β-galactosidase synthesis (Clark & Marr, 1964), while restriction of the rate of anabolism by the rate-limiting addition of the nitrogen or sulphur sources (Mandelstam, 1961; 1962), or by inhibiting protein synthesis with chloramphenicol (Paigen, 1963) or by removal of a required amino acid (McFall, 1961), will tend to repress β-galactosidase synthesis, presumably by the accumulation of catabolites.

It has been reported (Nakada & Magasanik, 1964) that catabolite repression causes a decrease in the rate of synthesis of messenger RNA specific for β-galactosidase, but it is not known whether the repressor defined by the

experiments of Pardee, Jacob & Monod (1959) mediates both inducer-sensitive induction-repression and catabolite repression. The fact that regulator constitutives (i^-) show repression of β-galactosidase synthesis in the presence of glucose has led to the belief (Cohn & Horibata, 1959; Clark & Marr, 1964; Mandelstam, 1962) that at least part of catabolite repression operates independently of the i-gene repressor, and to the postulation (Brown, 1961) of a second repressor for β-galactosidase in addition to the i-gene product. However, the serious objection can be raised that the absence of an i-gene product has not been shown in i^- constitutives. By analogy with the production of immunologically cross-reacting material (CRM) by β-galactosidase structural gene point mutants (z^-), it seems likely that most i^- point mutations would lead to an inactive i-gene product. It seems possible that this product might be partially activated by metabolites produced during glucose metabolism, giving the effect of catabolite repression. Loomis & Magasanik (1964) made an attempt to overcome this objection by observing glucose repression during the approximately one hour period of constitutive synthesis of β-galactosidase after transfer by conjugation of i^+z^+ into i^-z^- or lac-deletion recipients. However, one cannot be certain that the i-gene product was not present and merely activated by glucose metabolites during this period, since it is not known why i-gene expression in this situation is delayed compared with z-gene expression, as the two genes are very close to one another on the bacterial chromosome. The argument that the i-gene product must reach a certain cytoplasmic concentration before it can effectively repress enzyme synthesis may be countered by the suggestion that in the presence of glucose metabolites the i-gene product is effective at lower concentrations.

The ideal organism for answering the question of i-gene product involvement in catabolite repression would be one in which the i-gene was specifically deleted ($i^{del}o^+z^+$), but to our knowledge no such strain has been

isolated. Lacking this strain, we have compared under various conditions a series of strains separated by Steers, Craven & Anfinsen (1965), including an i^- point mutant and an o^c mutant carrying a deletion covering the i-gene and the operator, but leaving the z-gene intact. The existence of two phases of catabolite repression, an acute transient repression followed by a permanent less severe repression (Moses & Prevost, 1966), has permitted a detailed consideration of the relation between catabolite repression and the genetics of the lactose operon.

MATERIALS AND METHODS

Organisms. The strains of E. coli used are listed with their relevant genetics: 3000 ($i^+o^+z^+$), 3300 ($i^-o^+z^+$), o^c_{67} ($i^-o^c_{67}z^+$ [i and o deleted]), RV/F ($i^+o^+z^-$/F $i^-o^c_{67}z^+$)(all from E. Steers); 2000 - o^c ($i^+o^cz^+$)(from C. Willson); JC 14-2 and o^c_{67}-10, alkaline phosphatase constitutive mutants isolated from JC 14 ($i^+o^+z^+y^-$) and o^c_{67}, respectively, by the method of Torriani & Rothman (1961).

Experimental procedures. The measurement of CO_2 released from [$^{14}C_1$]glucose and [$^{14}C_6$]glucose has been described by Prevost & Moses (196). For experiments with alkaline phosphatase, cells were grown in glycerol-tris-mineral salts medium containing 0.7 mM-inorganic phosphate. All other techniques were unchanged from those used earlier (Moses & Prevost, 1966).

RESULTS

Effect of glucose on rates of β-galactosidase synthesis. Fig. 1 shows the effect of adding glucose (10 mM) on the synthesis of β-galactosidase in fully

induced strain 3000 (i^+o^+) and in strain 3300 (i^-o^+). In both cases the growth rate increased about 35% and a transient period of severe repression was observed after addition of glucose lasting 36-48 min., or 0.7 generations in each case. The differential rate of β-galactosidase synthesis after recovery from transient repression showed some fluctuation among different experiments, and was most often about 45% of the rate before glucose was added. In contrast, Fig. 2 shows the behavior of strains o^c_{67} (i and o deleted) and RV/F (essentially i^+ and o deleted). For both of these strains, in which the

increase in the growth rate on addition of glucose was normal, no transient severe repression followed glucose addition, but the differential rate of β-galactosidase synthesis was immediately and permanently repressed to about 45% of the earlier rate in the absence of glucose.

Strain 3300 was also tested for glucose repression in the presence of the inducer isopropyl-thio-β-D-galactoside (IPTG) to see if there would be any reversal of glucose repression analagous to the reversal by IPTG of fucose repression of an i^- constitutive reported by Williams & Paigen (1965). No effect of IPTG (5 mM) was observed on the growth rate, the length of the transient, or on the rates of β-galactosidase synthesis before, during or after the transient.

Fig. 3 shows the behaviour of strain 2000-o^c, a partial (9.6%) operator constitutive, in the presence and absence of IPTG. There was no effect of

the inducer on the percent repression during the transient, or on the length of the transient. The only difference between the two was the greater percentage degree of recovery from transient repression in the presence of IPTG.

Effect of glucose on tryptophanase synthesis. To check that mutational changes in the lactose operon affected catabolite repression only of the enzymes of that operon, the effect of glucose on the induced synthesis of L-tryptophanase was examined in strains 3300 and o^c_{67}. While quantitative differences in the rates of enzyme synthesis are often observed between different experiments, Fig. 4 shows no kinetic difference in behaviour between these two strains,

which differ markedly as far as ß-galactosidase synthesis is concerned.

Glucose metabolism in strains 3300 and o^c_{67}. It is known that transient catabolite repression can be abolished by a change in the pattern of glucose metabolism (Prevost & Moses, 196). Any study of the specific genetic correlations of the abolition of the transient for the lactose enzymes must therefore ensure that the abolition really is the result of a specific genetic change in the lactose operon and not due to an alteration of intermediary metabolism for some other irrelevant reason. It is obviously not easy to ascertain whether glucose metabolism is identical in two strains of an organism. Nevertheless, since the pentose phosphate cycle is known to be of particular significance with regard to catabolite repression, a test can be made to see whether there are any profound differences in this metabolic activity between the two strains.

In a pair of strains in which only one shows transient repression, and in which the genetics in the lactose operon are apparently identical, differences were observed in the respiratory release of $^{14}CO_2$ from [$^{14}C_1$]glucose and [$^{14}C_6$]glucose (Prevost & Moses, 196). Similar techniques applied to strains 3300 and o^c_{67} have shown little or no difference between the two as far as pentose phosphate cycle activity is concerned (Fig. 5).

Effect of chloramphenicol on β-galactosidase synthesis. Low concentrations of chloramphenicol, resulting in a moderate inhibition of growth, cause a very much more marked repression of β-galactosidase synthesis (Sypherd & Strauss, 1963). It was considered of interest to compare repression by chloramphenicol with repression by glucose reported above. Addition of chloramphenicol (1.27 µg./ml.) to cells growing exponentially in the glycerol medium and synthesizing β-galactosidase produced an immediate inhibition of the growth rate (which nonetheless remained exponential) and of the differential rate of enzyme synthesis. No further change was observed in the following 2 hr. In the presence of chloramphenicol the growth rate was typically 70% and the differential rate of β-galactosidase synthesis 25-45% of the rates in the absence of the inhibitor. Enzyme synthesis in strain RV/F was peculiarly sensitive to chloramphenicol with a differential rate of only 10% (Table 1).

Effect of glucose on alkaline phosphatase synthesis. A comparison of the response to glucose of β-galactosidase synthesis in strains o^c_{67} and RV/F with the response in strains 3000 and 3300 suggests that the transient part of the glucose repression is associated with a functional i-o regulator system, while the residual phase of mild repression after recovery from the transient does not require the presence or activity of these genes. It therefore seemed interesting to study the behaviour of alkaline phosphatase in these deleted mutants since alkaline phosphatase is an enzyme considered not subject to catabolite repression (McFall & Magasanik, 1960). Attempts were made to isolate an alkaline phosphatase constitutive mutant of o^c_{67} by selection on β-glycerophosphate in the presence of inorganic phosphate (Torriani & Rothman, 1961), but twenty such mutants isolated had all lost their entire

ß-galactosidase activity. The possible reason for this phenomenon will be discussed later. Lacking the desired mutant, a comparison was made of the two enzymes in two related strains: o^c_{67}, constitutive for ß-galactosidase, and o^c_{67}-10, constitutive for alkaline phosphatase and negative for ß-galactosidase. The level of alkaline phosphatase activity in o^c_{67}-10 suggests mutation of the R1 rather than the R2 regulator gene (Echols, Garen, Garen & Torriani, 1961).

Fig. 6 shows the effect of adding glucose on the synthesis of ß-galactosidase in o^c_{67} and alkaline phosphatase in o^c_{67}-10; both strains were growing in low-phosphate medium. In both strains glucose had no effect either on growth or on the rate of enzyme synthesis for 20 min. There was then a sharp change in rate in both cultures: the growth rate increased by 22-23% in each case and the differential rates of enzyme synthesis were repressed to 47% and 52% of their previous rates for ß-galactosidase and alkaline phosphatase, respectively. No transient was observed with either enzyme (cf. Fig. 2). One other strain, JC 14-2, has also been tested for the effect of glucose on the differential rates of synthesis of the two enzymes. In this case a transient was observed with ß-galactosidase, while alkaline phosphatase showed repression with no transient.

The 20 min. lag for glucose to affect growth and enzyme synthesis was in some way due to growth in low phosphate media. Strains which showed a growth lag in low phosphate media showed none when cultured in M63 medium which contained 0.1 M-inorganic phosphate.

Effect of chloramphenicol on alkaline phosphatase synthesis. The differential synthesis of alkaline phosphatase, like that of ß-galactosidase, is inhibited

by chloramphenicol. A comparison of the two enzymes in the related strains o^c_{67} and o^c_{67}-10 is shown in Table 2.

DISCUSSION

Transient repression is a sensitive way of measuring at least the most acute part of catabolite repression. While it has also been observed on occasion with L-tryptophanase and D-serine deaminase (Moses & Prevost, 1966), it is probably not really a "natural" phenomenon; it is obtained particularly with β-galactosidase because of the use of powerful gratuitous inducers which seem to be capable of causing partial reversal of catabolite repression after the most severe catabolic phase is past. The present study is concerned primarily with the influence of genetic factors on the expression of transient repression.

Glucose repression of β-galactosidase synthesis. The use of a series of regulatory mutants in the lac operon has permitted a genetic analysis of the factors governing the appearance of transient repression. A similar repression pattern was obtained in strains 3000 (induced) and 3300, showing that a change from i^+ to i^- does not by itself alter the response. This implies that, unlike weak inducers such as lactose, IPTG fully induces the cell so that in the presence of IPTG an i^+ cell behaves like an i^- one. This observation does not exclude the i-gene product (repressor) as a possible candidate for involvement in catabolite repression since in both induced 3000 and in 3300 repressor is not absent. In 3000 it has in some respects been altered by interaction with inducer, while in 3300 it has been altered by mutation, with physiologically similar consequences. The mutational change in 3300 seems

complete since IPTG had no effect in this strain either in the presence or absence of glucose.

Deletion of both the i and o genes, as in strain o^c_{67}, results in the total loss of the transient response, although the steady differential rate of enzyme synthesis in the presence of glucose was similar to that in 3000 and 3300 after recovery from the transient. We may thus conclude that transient repression is dependent on the presence of the i-gene or the o-gene, or both, but that the reduced final rate of enzyme synthesis in the presence of glucose is independent of these genetic factors. The results with RV/F, which differs from o^c_{67} in possessing a functional i-gene, the product of which is known to be able to affect the *trans* chromosome (Jacob & Monod, 1961), again showed no transient repression and the same final rate of enzyme synthesis as shown by the three previous strains. Since in the i-o gene regulatory system o^c is dominant to i^+ (Jacob & Monod, 1961), and because o^c_{67} is a complete operator constitutive (Steers *et al.*, 1965), one would expect no difference in behaviour between strains o^c_{67} and RV/F if the transient repression is dependent only on the i-o system, and indeed none was found. Further, since the only effective difference as regards the *lac* operon regulator genes between o^c_{67} and RV/F is the presence of i^+ in the latter compared with i^{del} in the former, we can be sure that the i-gene product has no role in transient catabolite repression unless the operator gene is functional; *i.e.* repressor can not act in catabolite repression at some site other than the operator. These observations, however, do not prove that the i-gene product *is* concerned in catabolite repression, but only that a functional operator gene (as in strains 3000 and 3300), or at least a partly functional operator (strain 2000-o^c), is required.

In strain 2000-o^c one might at first suppose that since this strain is about 10% constitutive by virtue of a mutation in the operator gene, leading presumably to a decreased affinity between the repressor and operator (Jacob & Monod, 1961), only the inducible part of β-galactosidase synthesis should show transient repression with glucose. Experimentally it was found that the uninduced synthesis also showed a transient (Fig. 3) and that, in fact, the differences between induced and uninduced synthesis were entirely quantitative, and constant proportionality was almost always maintained. The main significance of the findings with 2000-o^c compared with o^c_{67} is that even partial functionality in the operator gene is enough to permit transient repression. One may thus interpret (but not prove) the pattern shown by 2000-o^c as due to activation of the i-gene product during catabolite repression so that it now has a greater affinity for the operator, leading to a repression of enzyme synthesis. This could not happen in RV/F as in that strain the operator is believed to be totally deleted so that no degree of activation of the repressor could increase its interaction with the operator.

Glucose metabolism in strains showing different patterns of transient repression.

In order to be confident that the differences in the pattern of catabolite repression in strains 3000 and 3300 on the one hand, compared with o^c_{67} and RV/F on the other, are due to genetic differences in the lactose regulatory genes, it is essential to exclude the possibility that it is the glucose metabolism which became altered in these strains. As far as we know these four strains are closely related genetically and have been selected and isolated on the basis of the characteristics of their lactose genes. Nevertheless it is not inconceivable that other changes have taken place. The transient response in catabolite repression can be abolished by a change in glucose metabolism with no alteration of the lac operon (Prevost & Moses, 196), and a claim to have

isolated strains resistant to catabolite repression by virtue of a change in a regulatory system specific for this function (Loomis & Magasanik, 1965) has been criticized on the grounds that these mutants probably have an altered glucose metabolism (Moses & Prevost, 1966).

Three criteria have been used to test the similarity of glucose metabolism in two strains showing or not showing transient repression. By these criteria (effect of glucose on the induced synthesis of L-tryptophanase and on the rate of growth, and the respiratory release of $^{14}CO_2$ from glucose labelled in the first or the sixth carbon atoms) any differences in glucose metabolism between strains 3300 and o^c_{67} are trivial and not the origin of differences in catabolite repression responses.

Effect of chloramphenicol on β-galactosidase synthesis. Chloramphenicol, an inhibitor of protein synthesis, is known to set up a condition of catabolite repression when used at low concentrations sufficient to cause only a partial inhibition of growth (Sypherd & Strauss, 1963). We have observed that chloramphenicol (1.27 µg./ml.) has a similar effect on β-galactosidase synthesis in strains 3000, 3300 and o^c_{67}; in each case the growth rate was reduced to 70% while the differential rate of enzyme synthesis was reduced still further to 25-45% of the uninhibited rate. Thus chloramphenicol produced about as much repression as glucose in the phase following recovery from acute transient repression. Enzyme synthesis in strain RV/F is particularly sensitive to chloramphenicol, and the reason for this is not known.

Repression of alkaline phosphatase synthesis. Strain o^c_{67}, while exhibiting no transient repression, does show approximately the same final rate of β-galactosidase synthesis in the presence of glucose and chloramphenicol as do strains 3000 and 3300, and we are led to conclude that there are two types of catabolite repression: that requiring a functional operator gene, and that

independent of the operator (cf. Clark and Marr, 1964; Clark, 1965). The operator or i-o regulatory system is concerned only with acute transient repression; what, then, is the origin of the continuing mild repression after recovery from acute transient repression has occurred?

There is reason to suppose that this mild permanent repression may be neither specific for inducible enzymes nor truly a repression. An apparent repression, on a differential basis, would be produced if glucose stimulated an increase in the rates of synthesis of certain proteins during the growth shift-up, with a consequent dilution by default of proteins not so stimulated. Increases in growth rate are known to be associated with increases in the proportion of total protein to be found in the ribosomal fraction (Maaløe & Kjeldgaard, 1966), and one may surmise that the synthesis of enzymes specifically associated with glucose metabolism is also stimulated. β-Galactosidase, however, is an irrelevant enzyme as far as glucose metabolism is concerned, and therefore, apart from the specific repressive mechanism involving the operator gene, one might expect the rate of β-galactosidase synthesis to suffer by comparison with other proteins. Less is known about the metabolic consequences of chloramphenicol poisoning, though here too there is reason to suppose that a shift-up type of metabolic rearrangement occurs as evidenced by the rapid synthesis of RNA in the presence of chloramphenicol (Sypherd & Strauss, 1963). Fortunately a test may be attempted of this "dilution" hypothesis by studying alkaline phosphatase, another enzyme irrelevant to glucose metabolism, and one which is certainly not subject to any acute form of catabolite repression. In a strain (JC 14-2) showing a typical transient repression of β-galactosidase synthesis when glucose is added, with a later partial recovery, alkaline phosphatase synthesis showed inhibition without a transient acute phase. In comparing the strains o^c_{67} and o^c_{67}-10,

alkaline phosphatase synthesis in the presence of glucose is inhibited almost as much as the synthesis of β-galactosidase. One would expect chloramphenicol to have about the same effect on the synthesis of both enzymes, and Table 2 shows that this is indeed the case.

It was not possible to isolate an alkaline phosphatase constitutive mutant from strain o^c_{67} without also losing β-galactosidase activity. An explanation has been suggested by Dr. G. S. Stent (personal communication). In strain o^c_{67} both the operator and regulator genes of the lactose operon have been deleted (Fig. 7); it is not known how far the deletion extends beyond the i-gene. The structural gene (P) and the R1 regulator gene for alkaline phosphatase also lie beyond the i-gene. Judging by its level of alkaline phosphatase activity, o^c_{67}-10 is probably a constitutive mutant of the R1 regulator (Echols et al., 1961), and it is possible that this mutation is a partial deletion of the R1 regulator gene. In o^c_{67} the structural gene (z) for β-galactosidase may lie very close to the R1 regulator for alkaline phosphatase, so that partial deletion of the latter might readily overlap into the z gene (Fig. 7). The map of the E. coli chromosome published by Taylor & Thomas (1964) shows the R1 regulator gene on the far side of the structural gene from the lac operon, but we understand from Drs. A. Garen and M. Averner (personal communications) that the order of the R1 and P genes is not well established.

Conclusions. From the evidence and arguments presented in this paper it seems clear that in the lac operon acute transient repression is mediated via the operator gene and perhaps also via the product of the regulator gene. This may constitute an additional property of the Jacob & Monod (1961) regulation model: inducer would inactivate repressor and catabolite repression

would activate it even when (as in i^- strains) repressor had lost its normal affinity for the operator. Transient repression may be equivalent to "inducer-dependent repression" (Clark, 1965). Alkaline phosphatase is not sensitive to this type of repression.

Permanent, less severe, repression is not mediated via either the i-gene or the o-gene. It seems to be non-specific, and may not be a repression so much as a relative dilution through lack of specific stimulation. Alkaline phosphatase is also subject to this type of repression, which may be equivalent to "inducer-independent repression" (Clark, 1965).

The difference between acute transient repression and mild chronic repression is observed with the lactose enzymes when artificial inducers are used which are powerful enough to influence repressor in competition with catabolite repression. Inducer and catabolite corepressor probably do not act at the same site on repressor since i^- strains show transient repression as severe as in the related i^+ strains. With weak "natural" inducers the severe repression is probably prolonged indefinitely and no difference is seen between the two phases. In L-tryptophanase and D-serine deaminase synthesis in strain ML 30 the two phases are observed with glucose as the catabolite repressor and L-tryptophan and D-serine, respectively, as the natural inducers (Moses & Prevost, 1966). In this strain, however, the transient repression of β-galactosidase synthesis is unusually short and weak compared with the K-12 strains. All the strains used in the present study are derivatives of K-12.

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CAPTIONS FOR FIGURES

Fig. 1. Transient glucose repression of β-galactosidase synthesis in strains 3000 and 3300. Strain 3000 was induced with 0.5 mM-IPTG. Glucose (10 mM) added at arrows. Mass doubling times (min.) before and after glucose addition, and length of transient repression, respectively, for each strain: (a) 3000: 98, 75, 48; (b) 3300: 70, 51, 36.

Fig. 2. Glucose repression of β-galactosidase synthesis in strains o^c_{67} and RV/F. Glucose (10 mM) added at arrows. Mass doubling times (min.) before and after glucose addition, respectively, for each strain: (a) o^c_{67}: 81, 65; (b) RV/F: 84, 64.

Fig. 3. Glucose repression of induced and uninduced β-galactosidase synthesis in strain 2000-o^c. IPTG (0.5 mM) added at ↓; glucose (10 mM) added at ↑. Mass doubling times before and after glucose addition, and length of transient repression$_{\Lambda}^{(min.)}$, respectively: 95, 73 and 80 for both cases. (a) cells induced with IPTG; (b) uninduced cells.

Fig. 4. Effect of glucose on induced synthesis of L-tryptophanase in strains 3300 and o^c_{67}. L-Tryptophan (2.5 mM) was the inducer. Glucose (10 mM) added at arrows. Mass doubling times before and after glucose addition$_{\Lambda}^{(min.)}$, respectively: (a) 3300: 76, 56 (b) o^c_{67}: 67, 58

Fig. 5. Release of $^{14}CO_2$ from [$^{14}C_1$]glucose and [$^{14}C_6$]glucose by strains 3300 and o^c_{67}. The ratio of $^{14}CO_2$ evolved from [$^{14}C_1$]glucose to $^{14}CO_2$ from [$^{14}C_6$]glucose is plotted against time in min. after addition of labelled glucose to exponentially growing cells. The higher this ratio, in broad terms, the greater the proportion of glucose being oxidized via the pentose phosphate cycle. Metabolism entirely via glycolysis and the citric acid cycle would produce a ratio of 1. (a) 3300; (b) o^c_{67}.

Fig. 6. Comparison of effects of glucose on the synthesis of β-galactosidase in o^c_{67} and alkaline phosphatase in o^c_{67}-10. Glucose (10 mM) added at solid arrows; the growth rates increased at the points shown by the dashed arrows. Mass doubling times (min.) before and after glucose addition, respectively, for each strain: (a) o^c_{67}: 76, 62; (b) o^c_{67}-10: 89, 73.

Fig. 7. Possible genetic relation between strains o^c_{67} and o^c_{67}-10. Strain o^c_{67} differs from wild type by deletion of the o- and i- genes. o^c_{67}-10 may represent a further deletion including parts of z and R1. Abbreviations: AC, thiogalactoside transacetylase; y, galactoside permease; z, β-galactosidase; o, lac operator; i, lac regulator; R1, alkaline phosphatase regulator; P, alkaline phosphatase structural gene.

Table 1. Effect of chloramphenicol on the rates of growth and the differential rates of β-galactosidase synthesis in strains 3000, 3300, o^c_{67} and RV/F

Cultures of each strain, growing exponentially, were each divided into two parallel cultures to one of which was added chloramphenicol (1.27 µg./ml.). Growth by extinction and β-galactosidase activity was followed in each culture for 2 hr. Strain 3000 (inducible) was induced with IPTG (0.5 mM). Rates expressed on a relative basis.

Strain		3000	3300	o^c_{67}	RV/F
Growth rate:	- chloramphenicol	100	100	100	100
	+ chloramphenicol	71.0	70.7	68.7	73.8
Differential rate of β-galactosidase synthesis:	- chloramphenicol	100	100	100	100
	+ chloramphenicol	25.6	27.2	44.3	10.1

Table 2. Comparison of the effects of glucose and chloramphenicol on the differential rates of β-galactosidase and alkaline phosphatase synthesis in strains o_{67}^{c} and o_{67}^{c}-10, respectively

Exponentially growing cultures were treated with glucose (10 mM) or chloramphenicol (1.27 µg./ml.). The differential rates of enzyme synthesis are expressed as percentages of the corresponding rates before the addition of glucose or chloramphenicol.

	β-galactosidase (o_{67}^{c})	alkaline phosphatase (o_{67}^{c}-10)
Residual differential rates in the presence of glucose:	47.1%	51.5%
Residual differential rates in the presence of chloramphenicol:	44.3%	59.4%

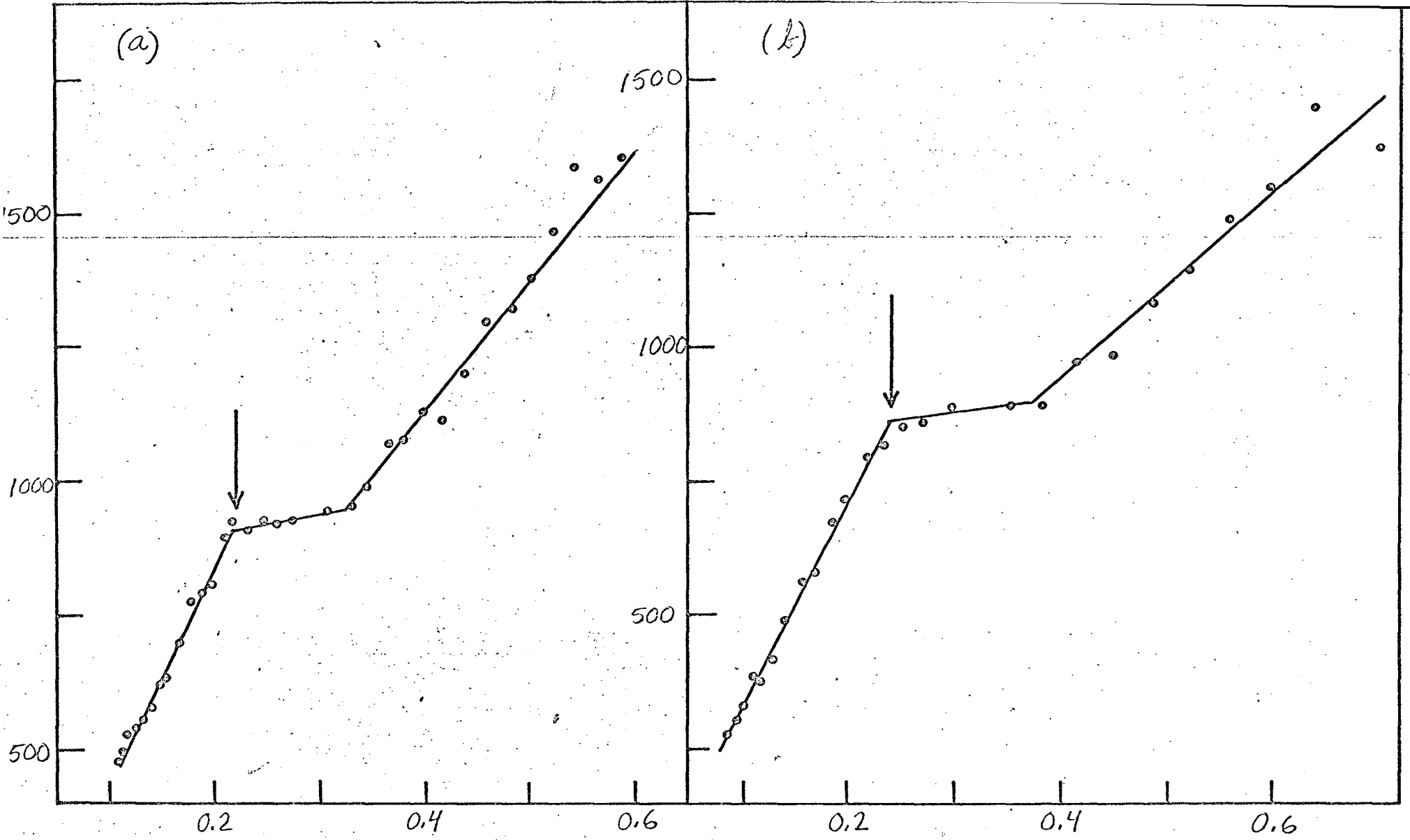

Fig. 1

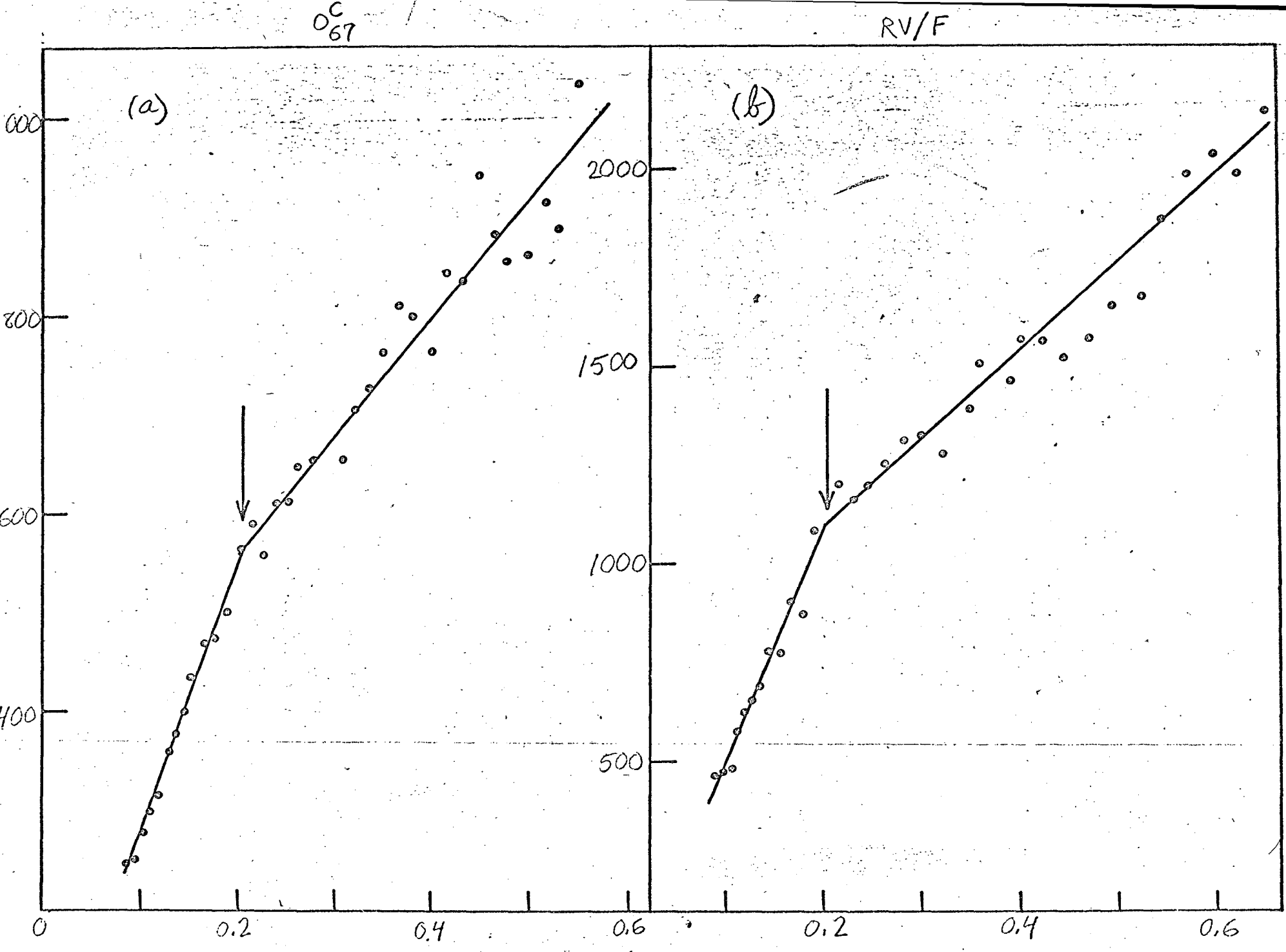

Fig. 2

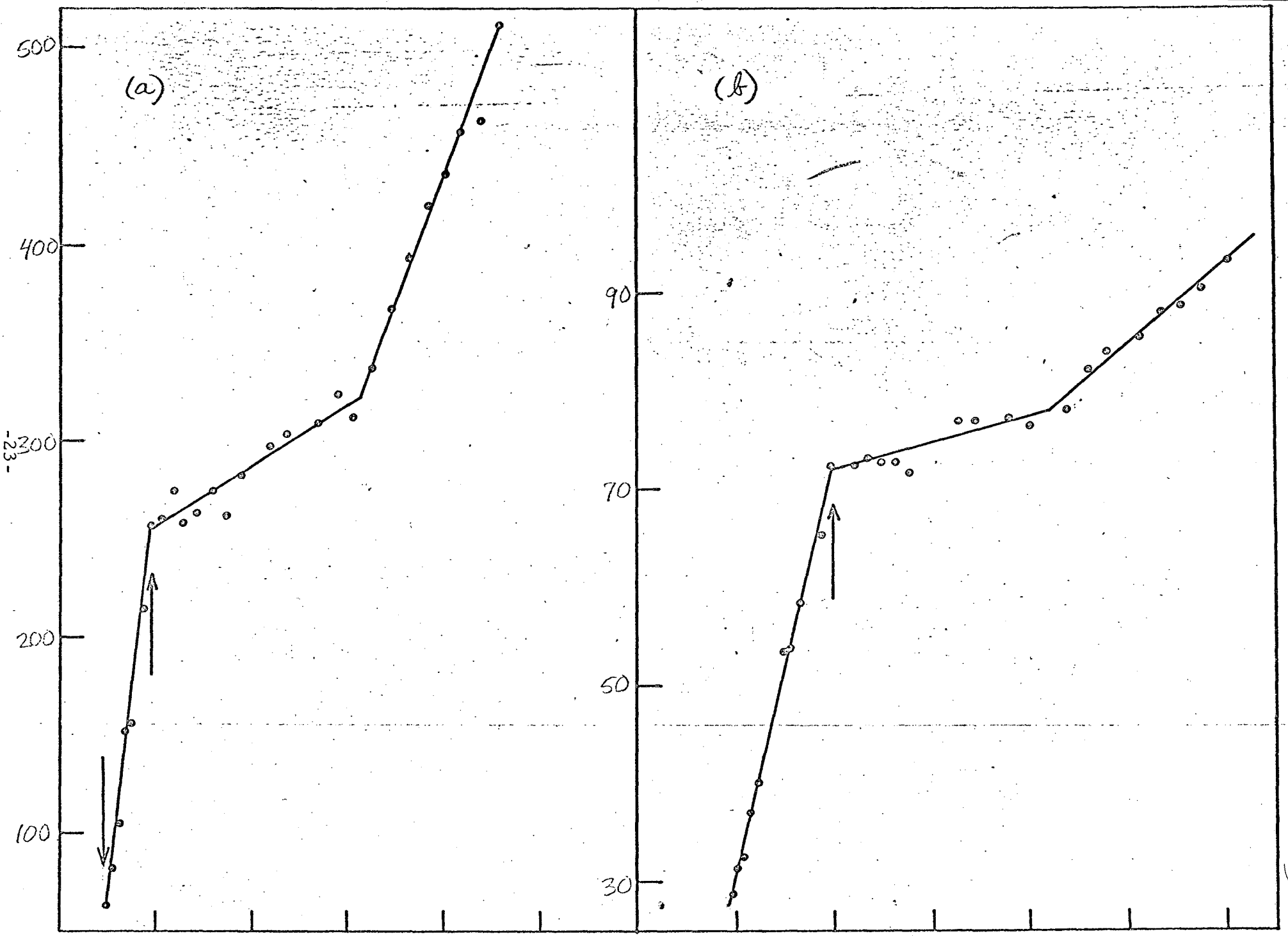

Fig.-3

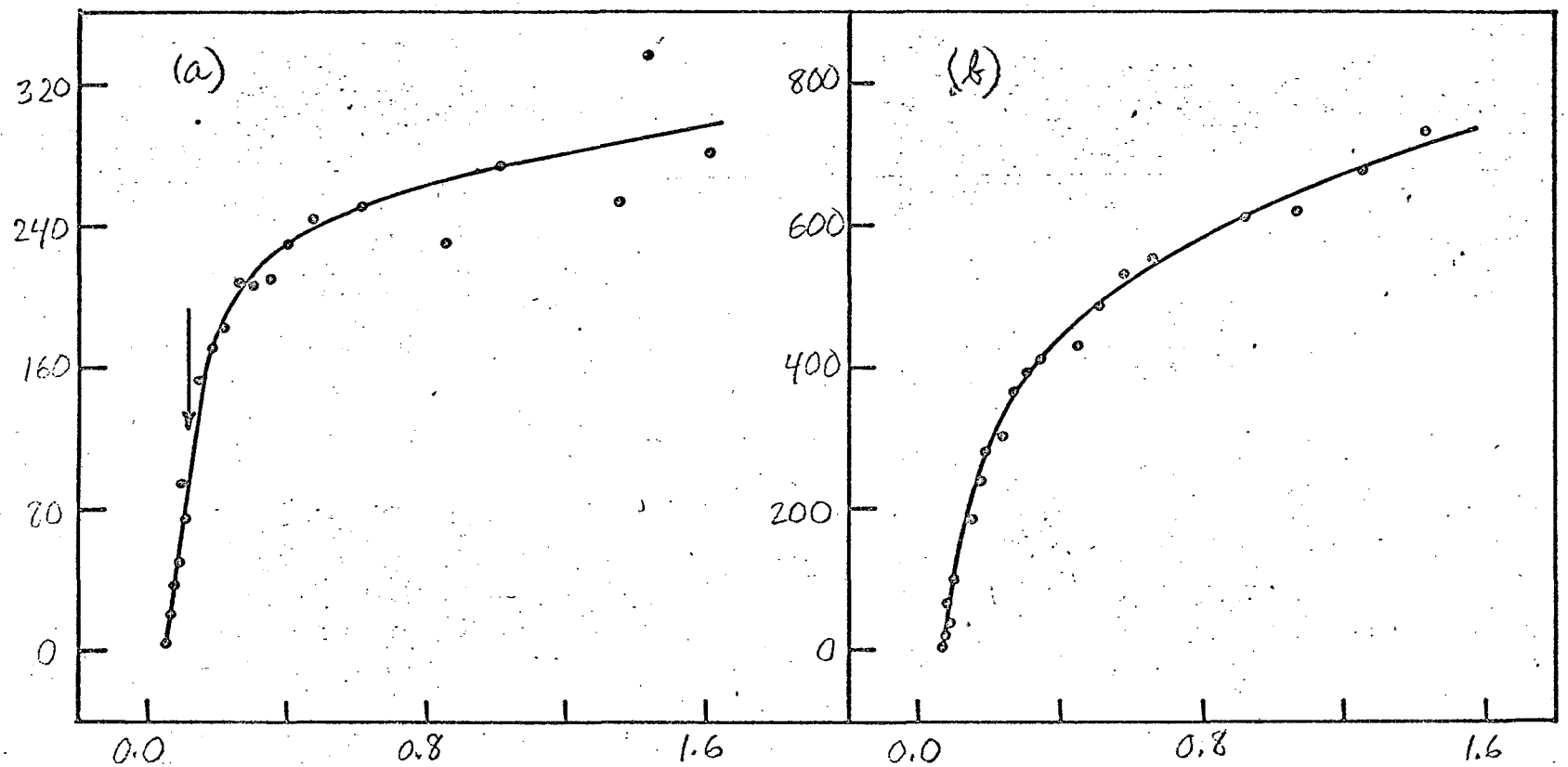

Fig. 4

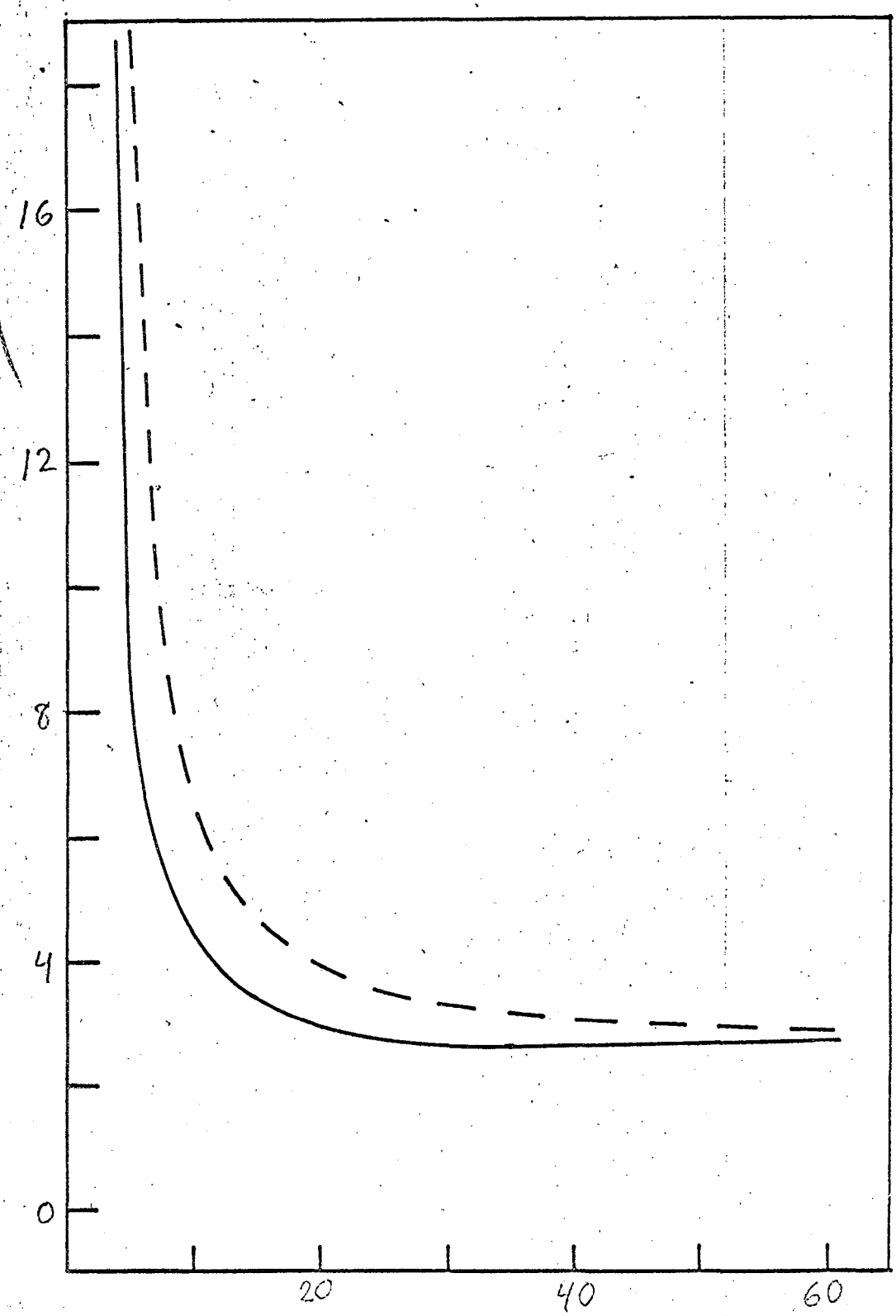

Fig. 5

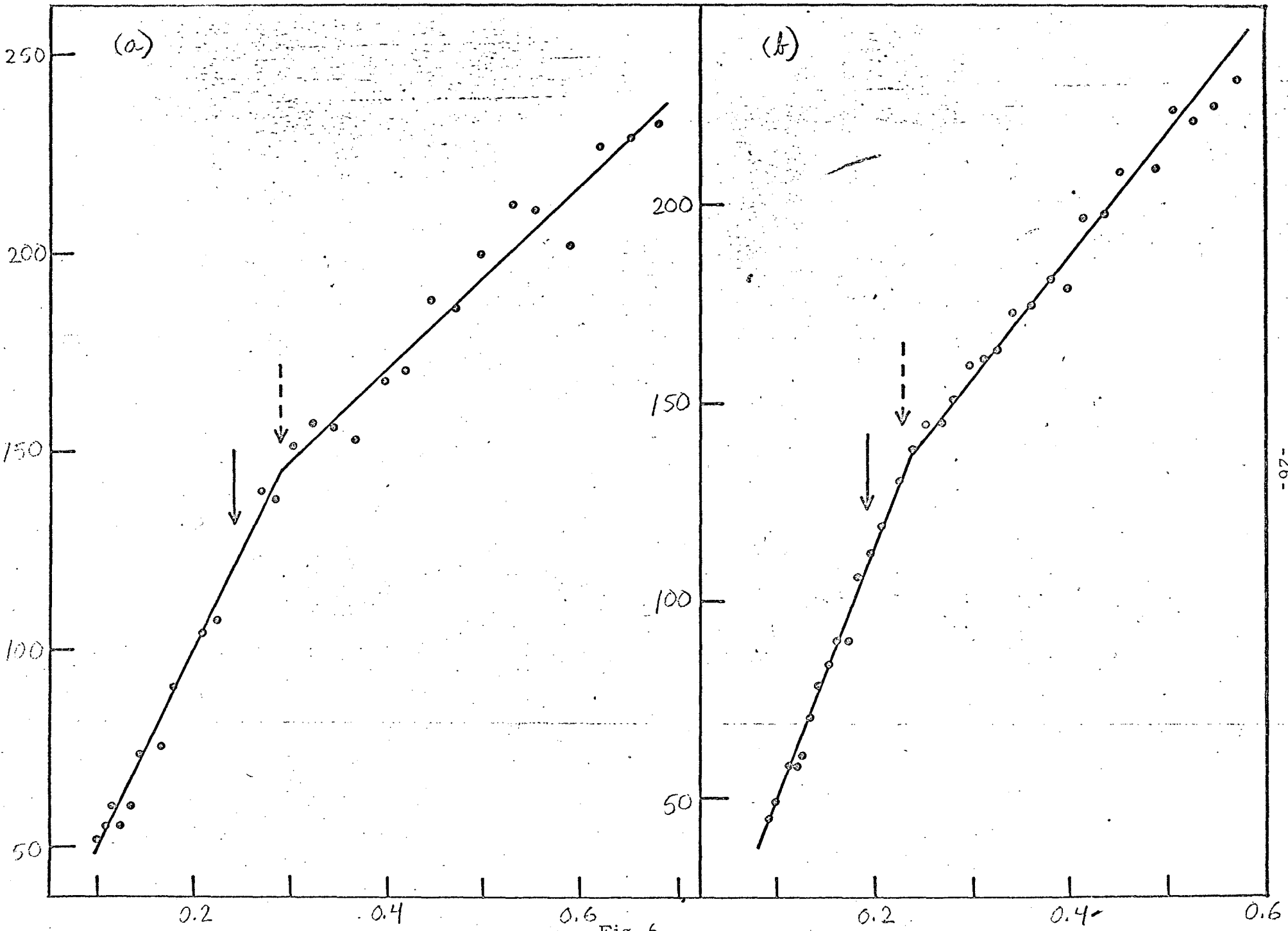

Fig. 6

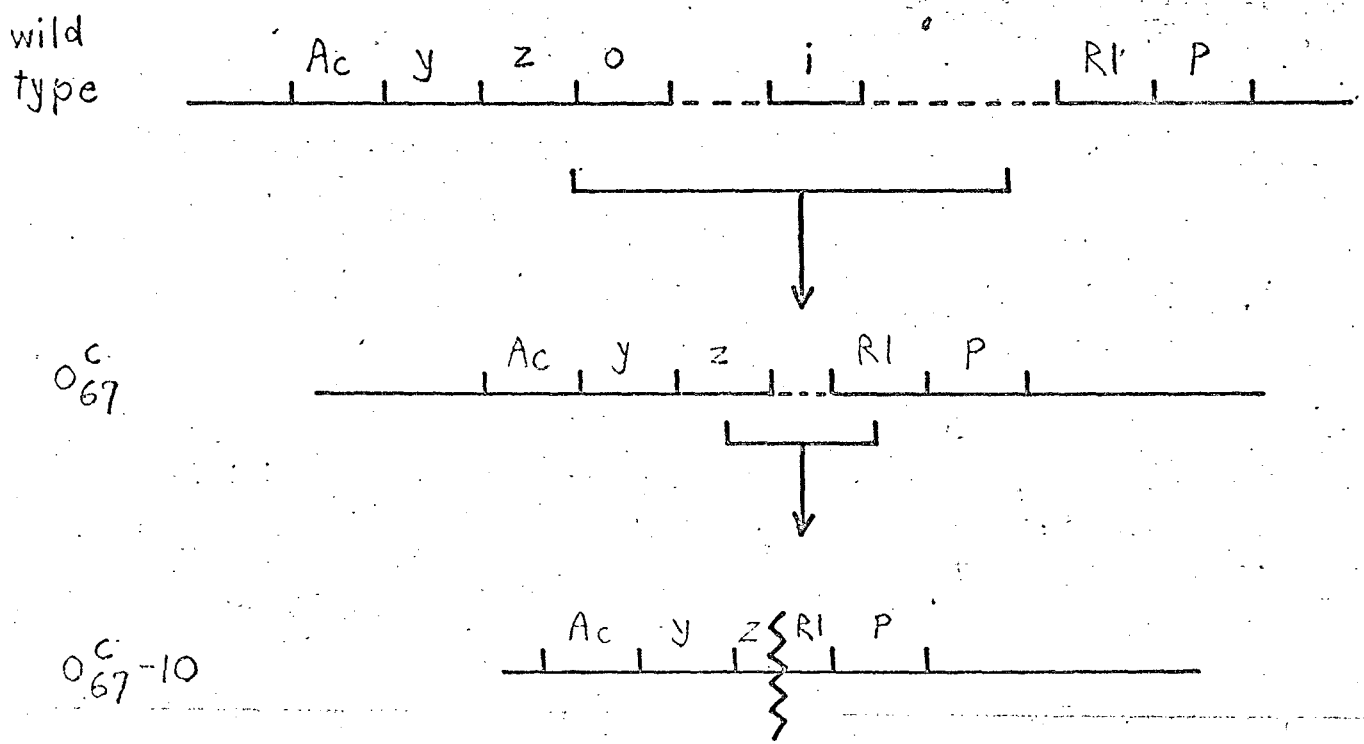

Fig. 7